**NOAA-NATIONAL OCEAN SERVICE**

**CENTER FOR COASTAL ENVIRONMENTAL HEALTH AND BIOMOLECULAR RESEARCH**

**FY03 SIGNIFICANT ACCOMPLISHMENTS**

**MARINE BIOTOXINS PROGRAM**

## IDENTIFICATION OF TOXIC BENTHIC DINOFLAGELLATES ASSOCIATED WITH CIGUATERA FISH POISONING FROM TEXAS OIL RIGS

Ciguatera-causing dinoflagellates are typically associated with coral reef ecosystems. The Texas coast has no coral reef systems except for the Flower Gardens National Marine Sanctuary well offshore and is generally considered a low risk area. However, oil production platforms are common along the coast and extend out past the edge of the continental shelf. The only reported cases of ciguatera in this area come from barracuda caught off these rigs. In collaboration with the University of Texas Marine Science Institute, we have investigated the population of epiphytic dinoflagellates from these oil production platforms. Samples examined using scanning electron microscopy contained the dinoflagellates *Gambierdiscus toxicus* and *Prorocentrum lima*; both species have been associated with ciguatera. A potentially new species of *Prorocentrum* has also been observed. Ciguatera appears to be endemic to the Texas coast, although at this time we cannot determine if the toxins are incorporated locally, or are being transported via fish migrations. Nonetheless, ciguatera remains a public health threat, since barracuda are commonly eaten in Texas and ciguatera symptoms are not commonly recognized.

## VOLUNTEER PHYTOPLANKTON MONITORING NETWORK EXPANDS FROM SOUTH CAROLINA TO GEORGIA

The South Carolina Phytoplankton Monitoring Network (SCPMN) began its third year with 38 groups monitoring over 50 sites along the coast of South Carolina. Volunteer groups are comprised of school students, volunteer citizen groups, and state park personnel. This community-based project of the Marine Biotoxins Program serves to increase the awareness of constituent groups about many issues related to harmful algae and directly involves volunteers in coastal stewardship. With the success of the program in South Carolina, our effort will be exported to selected sites in northern Georgia during fall 2003 through collaborations with the University of Georgia Marine Education Center and Aquarium. Observation and identification of phytoplankton along the South Carolina and Georgia coast will be useful in developing a species list and record of distribution patterns, as well as alerting NOAA scientists to the presence of potentially harmful species.

## SEQUENCING OF 8000 EXPRESSED GENES IN *KARENIA BREVIS* PROVIDES FOUNDATION FOR DEVELOPMENT OF DNA MICROARRAYS

*Karenia brevis* is a brevetoxin producing dinoflagellate responsible for red tides in the Gulf of Mexico, where it causes extensive fish kills, marine mammal mortalities, and human illness. Significant progress has been made towards understanding the factors controlling *K. brevis* blooms at the oceanographic level. However, little is known about the molecular and cellular controls that mediate responses of *K. brevis* to environmental cues leading to bloom formation and bloom termination. To facilitate research into the molecular biology of *K. brevis*, we embarked on a functional genomics project in FY01 with the development of a cDNA library as a gene discovery tool for identifying regulatory pathways present in this primitive eukaryote. This year, sequencing of ~8000 expressed sequence tags (ESTs) for genes expressed in the library has been completed and the sequences compared to the non-redundant GenBank sequence database using the basic local alignment search tool (BLAST). Oligonucleotide probes unique to each of the ESTs identified in the library are currently being designed for application to microarrays that will be developed in the next year. The DNA microarrays will be utilized to identify genetic pathways involved in bloom growth and toxicity.

**IDENTIFICATION OF STRESS PROTEINS IN *KARENIA BREVIS* MAY PROVIDE INSIGHT INTO MECHANISMS OF BLOOM TERMINATION**

Blooms of *Karenia brevis* initiate offshore and become a threat to coastal ecosystems and humans only when transported inshore by prevailing wind and oceanographic conditions. Once in coastal waters, *K. brevis* cells experience dramatic changes in their environment, resulting in temperature, salinity, light, turbulence, and oxidative stresses. The longevity of coastal *K. brevis* blooms depends on the degree to which they can adapt to environmental stresses encountered in coastal waters. Thus, understanding the physiological basis for adaptation (or failure to adapt) to the coastal environment is a prerequisite to understanding the mechanisms leading to bloom termination. Studies completed in FY03 identified for the first time a suite of stress proteins expressed in *K. brevis* and characterized their responses to heat, light, and oxidative stresses. This information will serve as the foundation for investigating mechanisms leading to dinoflagellate cell death and bloom termination.

**PURIFICATION OF A NOVEL TOXIC COMPOUND PRODUCED BY *ALEXANDRIUM MONILATUM* ISOLATED FROM THE GULF OF MEXICO**

Since the 1930's, the chain-forming dinoflagellate, *Alexandrium monilatum*, has formed blooms in coastal waters of all Gulf states and has frequently been associated with fish mortality events.. In 1967, research chemists described a cytotoxic, lipophilic compound derived from extracts of this species; however, the purification and structure of this toxic substance(s) was never reported. A strain of *A. monilatum* isolated from a red tide off Mississippi (strain AM01) was grown in batch culture to mid-log phase and examined for the possible production of bioactive compounds. The harvested cell mass was extracted using an elutropic series of increasing polarity using 5 different solvents and each fraction was tested for activity using different live animal and-cell based assays. Two distinctive toxic fractions were observed: a polar soluble fraction and a non-polar soluble fraction. Our current effort has focused on isolating and determining the chemical structure of the non-polar soluble compound, beginning with chromatographic separations. Subsequent mass spectral analysis using both LC-MS and MALDI-MS of the resulting purified compound yielded a molecular ion of 790 amu. Proton and carbon NMR structural analysis demonstrate a macrolide-like compound with four exo-cyclic double bonds. This novel compound has a nominal molecular formula of $C_{47}H_{98}O_8$.

**COLLABORATIVE STUDY WITH IRISH MARINE INSTITUTE PROVIDES NEW INSIGHTS ON THE MODE OF ACTION FOR AZASPIRACID, A NEW ALGAL TOXIN**

Azaspiracid (AZA) is a newly discovered algal toxin found recently in mussels from several northern European countries, including Ireland, UK, and Norway, and reported to have caused severe human intoxications in The Netherlands, Ireland, France, and Italy. Although AZA has been found to cause a range of severe effects in mammals, including tumor promotion, it remains uncertain as to how this toxin actually functions in the body. In order to address this issue, CCEHBR scientists have partnered with colleagues at the Irish Marine Institute to examine the mode of action for AZA. Our initial investigations have shown that AZA-1 is differentially cytotoxic to seven cell types, compromises cell membrane integrity, and alters cytoskeletal organization. These data suggest that AZA-1 has some unique properties among previously described marine toxins. Results of this work will be useful in designing bioassay tests for detecting AZA in seafood, identifying a treatment for the human illness associated with AZA exposure, and tracking this toxin in marine food webs.

**PRODUCTION OF BREVETOXIN BROUGHT TO HIGH ANALYTICAL PURITY PROVIDES COMPONENT NECESSARY FOR TOXIN DETECTION METHODS**

Analytically pure toxin standards are a critical component for toxin detection methods. Several methods commonly used to detect brevetoxins in shellfish and marine mammals require a radio-labeled form of brevetoxin. To produce this essential compound, brevetoxin must first be extracted from large batches of laboratory cultivated algae (approximately 400 liters of *Karenia brevis*). The demand for bulk purified toxin has lead to the development of a two stage toxin production method using preparative liquid chromatography-mass spectrometry. Fractions that meet the desired mass, chromophoric, and retention time characteristics are further subjected to nuclear magnetic resonance spectroscopy for purity analysis. This final quality assurance step ensures that contaminants not amenable to ionization are completely

removed and provides a much higher degree of accuracy in developing toxin reference and standard materials. The final product for radiolabeling will be sufficient to meet toxin detection needs of the research community for the next several years.

**SUCCESS OF PROTOTYPE REMOTE HARMFUL ALGAL BLOOM (HAB) SENSOR STIMULATES DEVELOPMENT OF SECOND GENERATION INSTRUMENT**

The first generation of an autonomous, *in situ* sensor for HAB species and their toxins, called the Environmental Sample Processor (ESP), has been field tested successfully for domoic acid-producing *Pseudo-nitzschia* and for saxitoxin-producing *Alexandrium*. As part of a collaborative project under the multi-agency National Oceanographic Partnership Program (NOPP), scientists from CCEHBR and the Monterey Bay Aquarium Research Institute (MBARI) are now designing a second generation ESP platform that will be smaller and include enhanced sampling/processing capabilities that will increase its flexibility for use in monitoring and research. Membrane-based arrays will provide concurrent, near-real time detection for both organisms and toxins. Data telemetered autonomously from the ESP to land-based facilities will aid in efforts to forecast bloom development and movement, and will ultimately be available through a web-based user interface.

**COLLABORATION WITH NAVAL RESEARCH LABORATORY SUCCESSFULLY TESTS PORTABLE BIOSENSOR FOR TOXIC ALGAE**

On-line, near-real time detection systems for harmful algal species and their toxins is a rapidly emerging field aimed at forecasting bloom development, persistence, and toxicity as well as providing data to facilitate rapid and more effective responses to harmful algal blooms. Recently the Naval Research Laboratory demonstrated that cultured neuronal networks grown over microelectrode arrays (MEAs) are capable of detecting brevetoxins, saxitoxin, and domoic acid. An on-site collaboration at the Marine Biotoxins Program using a prototype portable battery-operated unit containing a central core of living neurons growing on a biosensor chip sought to determine if the sensor could detect these toxins directly in the seawater growth medium of *Alexandrium fundyense* and *Karenia brevis* The instrument responded with positive toxin signatures from the sonicated medium of each red tide alga, but not from non-toxic isolates of the same algal genus. This successful trial provided evidence that the prototype MEA has the capacity to detect toxins associated with cells of toxic algal species and exhibits the potential for monitoring toxin levels during harmful algal blooms.

**OPTIMIZATION AND INTER-LABORATORY COMPARISON OF PSP RECEPTOR ASSAY COMPLETED WITH CALIFORNIA DEPT. OF HEALTH SERVICES**

The technology for a rapid, cost-effective receptor binding assay for paralytic shellfish poisoning (PSP) toxins developed by CCEHBR scientists was transferred to colleagues at the California Department of Health Services (CDHS). Due to differences in instrumentation between the two laboratories, optimization of the assay at CDHS was required, which was followed by an inter-laboratory comparison of results. Precision, accuracy, and sensitivity (LOD = 0.2 µg STX eq./100 g shellfish tissue) of the CDHS-modified protocol were equivalent to those of the original method. Determination of PSP toxin concentrations in shellfish samples collected through the CDHS monitoring program agreed closely between the two laboratories (within ~10%), demonstrating the robustness and adaptability of the assay. This technology shows a very high potential for replacing the currently employed mouse bioassay (MBA), which has drawn increasing criticism due to its use of live animal testing. At present, the receptor assay can serve as a rapid, high throughput screen prior to testing by MBA and provide an early warning of increasing PSP toxicity when toxin levels are below the MBA limit of detection.

**CHARACTERIZATION OF PSP TOXIN TROPHIC TRANSFER THROUGH ZOOPLANKTON GRAZERS IN THE GULF OF MAINE**

The initial vectors involved in transferring PSP toxins from their algal producers to higher trophic levels are important in determining the ecosystem components ultimately affected by these potent neurotoxins. Since zooplankton frequently serve as this initial vector, CCEHBR scientists, in collaboration with

colleagues at U. Mass. Dartmouth and the Woods Hole Oceanographic Institution, are studying entry of PSP toxins into the grazer community in order to identify the primary routes of trophic transfer in the Gulf of Maine. Results to date indicate that PSP toxins can accumulate in grazer size fractions ranging from 64 to >500 µm, with the distribution of toxin changing as a function of the zooplankton species present and their abundance, as well as the amount of toxin contained within the *Alexandrium* cells being grazed. PSP toxin entry into the larger zooplankton size fractions was observed during late spring/early summer, which has important implications for making these toxins available to cetaceans that actively feed on copepods in this region over the same time period.

**COLLABORATIVE STUDY WITH THE INSTITUT LOUIS MALARDÉ PROVIDES COMPARATIVE CIGUATOXIN ANALYSIS IN SEVERAL SPECIES OF PACIFIC REEF FISH**

Ciguatera annally affects as many as 50,000 people worldwide, posing a significant public health threat and an enormous economic challenge especially in tropical islands. Therefore, a critical need exists for the development of a rapid and sensitive screening test for the detection of ciguatoxin. A collaborative study with the Institut Louis Malardé investigated whether whole blood sampled on blood collection cards could substitute for the more demanding use of fish tissue extracts in the receptor binding assay for the screening of ciguatoxin exposure in fish. We have determined the concentration of ciguatoxins in dried-blood spot specimens from five fish species caught in French Polynesia and compared these concentrations to flesh toxicity using a receptor assay. Blood concentrations for ciguatoxins (0.30-0.54 ng/ml P-CTX-1 equivalents) closely correlated with toxin content in the flesh. With improvement of detection limits, this approach has the potential to be a useful procedure for fish screening, environmental risk assessment, or clinical diagnosis of ciguatera fish poisoning in human or marine mammals.

**DOMOIC ACID-INDUCED GENE EXPRESSION IN MOUSE BRAIN IDENTIFIED BY DNA MICROARRAY TECHNOLOGY**

Domoic acid is responsible for amnesic shellfish poisoning in humans and is the causative agent of extensive marine mammal mortalities. Domoic acid is an excitatory neurotoxin that mimics the neurotransmitter glutamate and is a potent activator of certain subtypes of brain glutamate receptors. Persistent activation of these receptor subtypes results in calcium dependent cell death and neuronal lesions in areas of the brain where these receptors are heavily concentrated. To better understand the mechanisms involved in the response to toxic levels of domoic acid in mammals, we have employed microarrays to characterize global gene expression profiles in the mouse brain following acute domoic acid exposure. Approximately 2-2.5% of all the genes expressed in the mouse brain undergo a significant change in expression following domoic acid exposure. Some of the early induced genes include those involved in calcium homoestasis as well as pain and inflammatory responses. Down regulated genes include transcription factors involved in expression of signaling pathways activated by domoic acid. Understanding the coordinated expression and interaction of genes following acute toxicity will provide a better understanding of the mechanisms of neurotoxicity caused by marine algal toxins and mechanisms of neuroprotection. Comparative studies of gene expression profiles in response to different algal toxins are anticipated to yield biomarkers of exposure.

**FIRST TIME IDENTIFICATION DOMOIC ACID IN WHALE MORTALITY EVENT ON GEORGES BANK EXPANDS THE THREAT OF MARINE TOXIN EXPOSURE TO NORTH ATLANTIC**

Between June 17 and July 30, 2003, twelve endangered humpback whales, one pilot whale, and one fin whale were found dead in open waters off Massachusetts near Georges Bank. Tissues and fluids collected from the whales by the NMFS Marine Mammal Stranding Network were tested by the Marine Biotoxins Analytical Response Team for the presence of saxitoxin and domoic acid, two algal toxins known to occur in these waters and previously associated with marine mammal mortality events. Saxitoxin was found at low concentrations in the stomach contents and feces of one humpback whale, while all other samples were negative. The presence of saxitoxin indicates exposure of the whale to this neurotoxin; however, concentrations present were lower than those recorded previously in actively feeding right whales. Saxitoxin is responsible for paralytic shellfish poisoning in humans and is suspected in the 1988 die-off of

humpback whales in Cape Cod Bay. Domoic acid was confirmed in the intestinal contents of a different humpback whale at a level similar to those previously documented in the feces of fatally intoxicated marine mammals. Frustules of diatoms from the domoic acid producing genus, *Pseudo-nitzschia*, were also found in the intestinal contents of this animal. Domoic acid is a neurotoxin that causes seizures and permanent brain damage and has been responsible for sea lion, otter, and cetacean mortalities along the California coast. Domoic acid has been identified previously on Georges Bank associated with scallops, leading to closure of that fishery in the mid-1990's. This is the first documentation of domoic acid associated with a marine mammal mortality event in the northeastern U.S. and provides another line of evidence that the range of harmful algal bloom impacts may exceed current estimates.